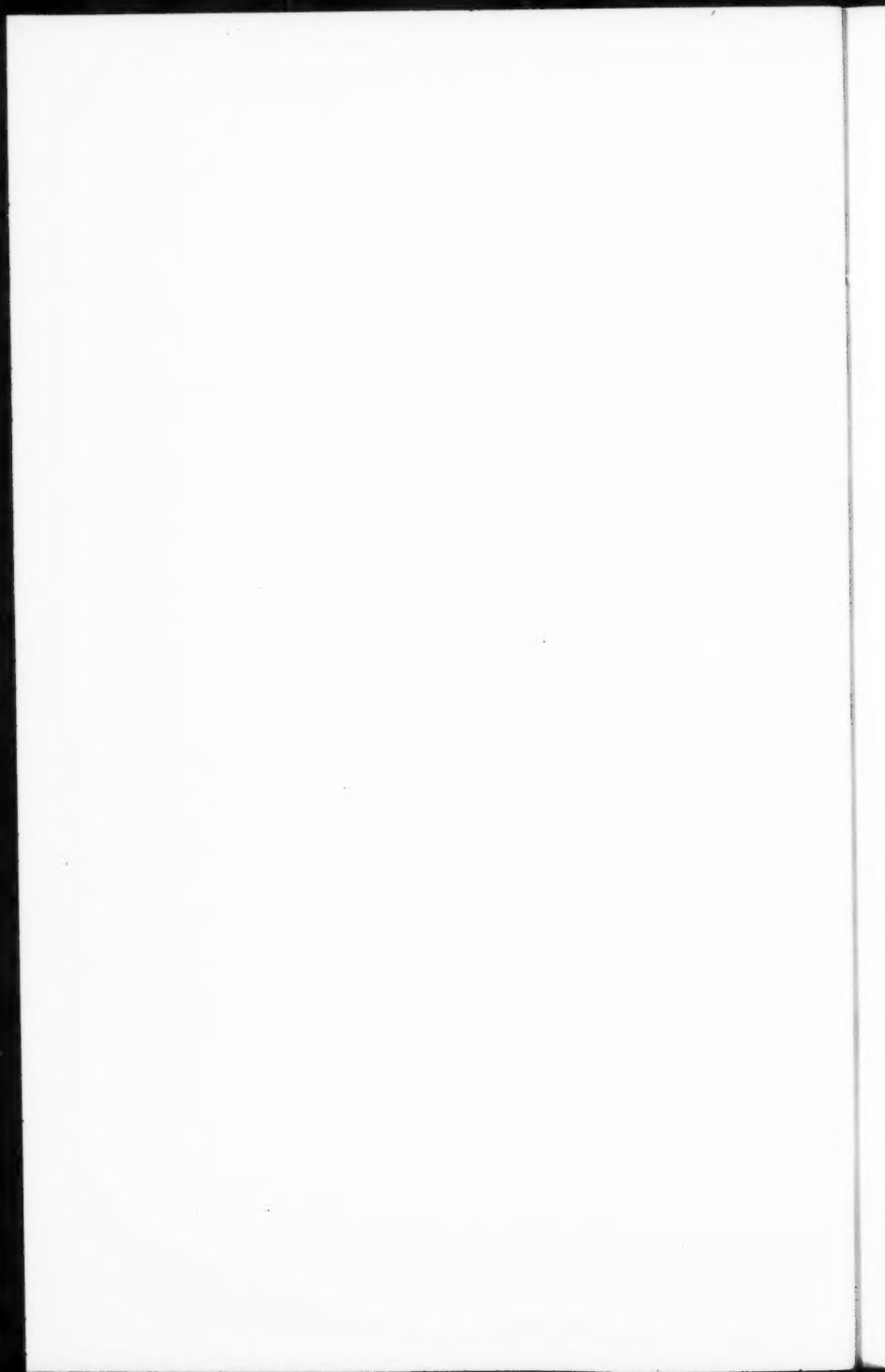


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BLOOD PHOSPHATASE
A COMPARISON OF THE BODANSKY, JENNER
AND KAY, AND KING METHODS*

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The appearance of the term "blood plasma phosphatase" in recent (1930-1934) medical literature and the demand for phosphatase determinations as diagnostic aid in cases of bone disease (Kay¹⁻², Roberts³) and jaundice (Roberts⁴, Armstrong, King and Harris⁵) makes a discussion of the subject and a comparison of accepted methods appropriate at this time.

Phosphatase, or perhaps more rightly phosphoesterase, is an enzyme having the specific power to hydrolyze certain phosphoric esters. Phosphoesterases with variable properties have been demonstrated in the plasma and cells of blood and in extracts of muscle, growing bone and cartilage, and in renal tissue, and, in lesser strength, in other organs.

While it has been known that the numerous phosphoric esters so widely distributed in animal and plant tissue were hydrolyzed by tissue extracts, little was known of the enzymes (phosphatases) until Robison (6) in 1923 studied the various enzymes in connection with his work on hexosmonophosphate. Since, individual phosphatases have been isolated and found to have characteristic properties. With some the optimum pH varies, some hydrolyze only certain phosphoric esters, and some differ in activity.

* Read before the Third Annual Convention of the American Society of Clinical Laboratory Technicians, Atlantic City, New Jersey, June 10-12, 1935.

The first phosphatase activity in blood was demonstrated by Martland, Hansman and Robison (2). They showed that plasma would slowly hydrolyze a portion of the phosphoric esters of red blood cells contained in a protein free filtrate from whole blood. Shortly afterward Shipley (6) observed that when slices of the cartilage and bone of rachitic rats were placed in serum of normal rats, calcification occurred in a similar manner to that taking place in a living rat fed on a curative diet. Earlier, Grosser and Husler (6) found that many mammalian tissues would hydrolyze glycerophosphate, but were unable to demonstrate this activity in blood. Other workers (2) reported little or no activity.

Kay (7) reported plasma activity in hydrolyzing phosphoric esters relatively weak as compared to bone, intestinal mucosa and the kidney. These three tissues are the most active and quantitatively important as far as phosphatase is concerned; the liver and central nervous system is relatively low and the muscles still less. He advances the theory that in diseases of phosphatase rich tissue, particularly conditions in which there is widespread breakdown of those tissues, with possible leakage of intracellular materials into the blood, an increase above normal might occur in plasma. This theory is substantiated by the fact that all workers have reported marked increases in plasma phosphatase in disease of the bones such as osteitis deformans, rickets, osteitis fibrosa, osteomalacia; this increase being as much as four to twenty times normal (1, 3, 7, 8, 9, 10).

Blood phosphatase possesses the following properties: Has been found to hydrolyze all the phosphoric esters which have so far been tried (2). These have been hexosdiphosphate, synthetic alpha and beta glycerophosphate, pyrophosphate, glycerophosphate and guanine nucleotide. The optimum pH for action on glycerophosphate is between 8.8 and 9.1 under the conditions of the substrate described by Kay with glycine-sodium hydroxide buffer. It is the same for normal human, rat or rabbit plasma and for plasma in cases of bone diseases in man. Magnesium ions act as a powerful stimulant to activity, while calcium ions act as a mild inhibitor.

The demand for phosphatase determinations will come through the appreciation of the fact that it is the belief of many investigators that phosphatase of the blood offers a more delicate indication of abnormal calcium-phosphorous metabolism than determinations of those elements or even x-ray can offer. Smith and Maizels (11, 12, 13) working with rickets, scurvy and fractures, found that the calcium and phosphorous determinations were not true criteria of the presence or absence of rickets. In all their work they found the phosphatase remaining high for weeks after the calcium and phosphorous had returned to normal and x-ray showed normal bone

formation. Also they found that the phosphatase changes occurred before the disease had progressed enough to note radiological changes.

Results show that the phosphatase is invariably high in obstructive jaundice as that resulting from stone in the common duct or carcinoma of the head of the pancreas. In all other types, the figures are normal or only slightly elevated even though the patient is deeply jaundiced (4, 14, 15). There is no relationship between the phosphatase and van den Bergh figures. Workers feel that phosphatase may be used to differentiate various types of jaundice.

Three methods of determination of phosphatase activity will be described in detail and a comparison made of the advantages and disadvantages and results obtained in our laboratory.

Jenner and Kay Method

Kay (2) was one of the first workers to publish a method for the determination of phosphatase in plasma. At best it was very cumbersome and badly suited to clinical uses. Jenner and Kay modified the original method to adapt it to clinical work. The modification consisted of reducing the time of hydrolysis from forty-eight to three hours; the plasma requirement reduced from five ml. to one or two ml.; filtration of the centrifuged plasma eliminated and pH determinations on the buffered substrate eliminated. This new method is slightly less precise than the original but more practical and suitable for clinical work.

In brief the method consists of the hydrolysis of an excess of sodium beta glycerophosphate and the resulting inorganic phosphorous determined. The activity of the phosphatase is calculated by the difference between the inorganic phosphorous thus determined and that of the original serum.

SOLUTIONS AND REAGENTS.

Sodium beta-glycerophosphate substrate. (Obtainable from Boots Pure Drug Co., Nottingham, England, or Eastman Kodak Co., Rochester, New York.) 2.5 gm. of pure crystals are dissolved in 100 ml. of distilled water. Keep in refrigerator with 3 or 4 drops of chloroform.

Glycine-sodium hydroxide buffer solution. Analytically pure glycine 6.06 gm. and 4.68 gm. of pure sodium chloride are dissolved in 328 ml. sodium hydroxide, N/10 in a 1 liter volumetric flask. Dilute to volume with distilled water and mix.

Standard substrate solution. Mix one part of sodium glycerophosphate substrate and five parts of glycine-sodium hydroxide buffer solution. May be kept in the refrigerator with 2 or 3 drops of chloroform.

Trichloroacetic acid solution, 15%. Dissolve 15 gm. of chemically pure trichloroacetic acid in sufficient distilled water to make

100 ml. of solution.

Sulphuric acid solution, 10N. Add 282 ml. of concentrated sulphuric acid to about 500 ml. of distilled water. Cool and dilute to 1 liter.

Sodium molybdate solution, 7.5%. Dissolve 7.5 gm. sodium molybdate Kahlbaum's "zur analyze" or other pure product in sufficient distilled water to make 100 ml. of solution.

Molybdic acid mixture. Mix one part of sulphuric acid solution, 10N with two parts of distilled water and one part of sodium molybdate solution, 7.5%.

Stannous chloride solution, stock. Dissolve 40 gm. of stannous chloride in sufficient concentrated hydrochloric acid to make 100 ml. of solution. Keep in a dark colored bottle in a dark place.

Stannous chloride solution, dilute. Dilute 1 ml. of stannous chloride solution, stock to 200 ml. with distilled water. Prepare daily.

Phosphate standard solution, stock. Dissolve 0.4390 gm. of pure dry mono-potassium phosphate (KH_2PO_4) in distilled water in a 1 liter volumetric flask. Dilute to volume and mix. 1 ml. contains 0.1 mg. P.

Phosphate standard solutions, dilute. Prepare three dilutions of the phosphate standard solution, stock by diluting 1, 2 and 3 ml. to 50 ml. respectively in a 50 ml. volumetric flask with distilled water. 5 ml. of each contains 0.01 mg., 0.02 mg. and 0.03 mg. respectively.

METHOD.

Centrifuge blood to obtain the plasma or serum. Pipette off plasma and recentrifuge.

Place 5 ml. of standard substrate solution in each of four clean 15 ml. test tubes. Two tubes are marked "control" and two "unknown A and B."

Dilute 2 ml. of plasma with 2 ml. of 0.9% sodium chloride solution and add 0.5 ml. of this diluted plasma to each of the above tubes. Mix by gentle inversion. In cases of suspected generalized bone disease, the plasma may be diluted to 1 to 5 or even to 1 to 10 with 0.9% sodium chloride solution (giving plasma values used of 0.05 and 0.025 ml. respectively) and run along with the standard dilution.

The "unknown" tubes are stoppered and placed in a water bath at 38° C. for exactly three hours. To each "control" tube add 2 ml. of 15% trichloroacetic acid solution, stopper, mix and allow to stand for ten minutes. Filter through a small (7 cm.) filter paper (Whatman No. 40 or other phosphate free paper) and set filtrates aside until "unknown" tubes are ready. After three hours the "unknown" tubes are removed from the water bath and cooled rapidly under

cold water. Then add 2 ml. of 15% trichloroacetic acid solution to each, mix, let stand for ten minutes and filter as above.

Place 5 ml. of each of the above filtrates into clean labeled test tubes and prepare two "standard" tubes. Place 5 ml. of phosphate standard solution, dilute No. 1 (5 ml. contains 0.01 mg. P) in one and 5 ml. of phosphate standard solution, dilute No. 2 (5 ml. contains 0.02 mg. P) in the other.

To all tubes add 4 ml. of molybdic acid mixture.

To all tubes add 1 ml. of freshly prepared stannous chloride solution, dilute, *stirring while adding*. (Use a small, clean glass rod with a bent tip. Drain and rinse with distilled water between each stirring.) The final volume should be kept within the limits of 10 plus or minus 0.1 ml.

Allow to stand for three minutes and read in the colorimeter against the nearest matching standard. (Errors begin to arise if the color of the "unknown" varies from the standard by more than 40%.)

Calculate according to the formula

$$\frac{Rs \times Cs \times l \times 100}{Ru} \times \frac{0.167}{1} = \text{mg. P per 100 ml. plasma.}$$

Phosphatase units = $\frac{(\text{mg. P per 100 ml. unknown plasma}) - (\text{mg. P per 100 ml. control plasma})}{1}$

1 unit Kay equals the equivalent of 1 mg. inorganic phosphate (expressed as P) liberated from an excess of sodium betaglycerophosphate in three hours at 38° C. and a pH of about 8.6, per 100 ml. of plasma. Normal values—3.2 to 7.9 units.

Bodansky Method

Bodansky (10) first published a method for the determination of phosphatase activity in 1931. This first method, while essentially the same as the Jenner and Kay method just described, modified that method principally by cutting the incubation time to two hours and using monosodium-diethyl-barbiturate (veronal) as a buffer. Two years later, 1933, he published a revision of this method, adding to the accuracy and convenience in several ways by:

1. Use of serum instead of plasma.
2. Use of combined solution of buffer and substrate of proven keeping qualities.
3. Reduction of time of incubation to one hour or less.
4. Simplification of calculations by changing the final dilution of serum in the "total inorganic phosphate" filtrate from 16 to 20.
5. Corrections for analytical errors in the presence of glycerophosphate and trichloroacetic acid and also for deviation from Beer's law.

This method is basically the same as the Jenner and Kay.

SOLUTIONS AND REAGENTS.

Buffered substrate solution. Dissolve 2.5 gm. sodium beta-glycerophosphate and 2.12 gm. monosodium-diethyl-barbiturate (veronal) in distilled water in a 500 ml. volumetric flask and dilute to volume. Mix thoroughly. Keep in refrigerator after adding 2 or 3 drops of chloroform or toluene.

Trichloroacetic acid solution, 10%. Dissolve 10 gm. chemically pure trichloroacetic acid in sufficient distilled water to make 100 ml. of solution.

Sulphuric acid solution, 10N. Same as for Jenner and Kay (see above).

Sodium molybdate solution, 7.5%. Place 90 gm. molybdc acid (ammonia and phosphate free) in a 2 liter volumetric flask. Add 250 ml. of sodium hydroxide, 5N. When the molybdc acid is dissolved dilute to volume with distilled water and mix. The solution should be faintly alkaline to phenolphthalein. Let stand and decant for use. This solution is preferred to that used by Jenner and Kay because the latter has frequently given blue blanks.

Molybdc acid reagent. Take one volume of cold sulphuric acid, 10N in a suitable flask. Add quickly while mixing, one volume of sodium molybdate solution, 7.5%. Dilute with two volumes of distilled water. Prepare daily.

Stannous chloride solution, stock. Prepare 60% solution by dissolving 30 gm. of stannous chloride, cp. quality, in concentrated hydrochloric acid in a 50 ml. volumetric flask and diluting to volume with more acid. Keep in refrigerator for about a month.

Stannous chloride solution, dilute. Dilute 1 ml. of stock solution to 200 ml. Prepare daily and keep in glass stoppered bottle.

Phosphate standard solution, stock. Dissolve 0.4390 gm. pure mono-potassium phosphate (KH_2PO_4) in distilled water in a 1 liter volumetric flask. Add 1 ml. of concentrated sulphuric acid and dilute to volume. Mix thoroughly. 10 ml. contains 1 mg. P.

Phosphate standard solution, dilute. Dilute 10 ml. of phosphate standard solution, stock to 250 ml. in a volumetric flask. Preserve with a drop of toluene. 5 ml. contains 0.02 mg. P.

METHOD.

Prepare serum by centrifuging twice.

Place 10 ml. of substrate solution into each of two glass stoppered test tubes (18 x 150 mm.) Avoid aeration of the solution.

Place in a water bath at 37° C. for a few minutes.

Add 1 ml. of prepared serum to each tube, mix by a single inversion and replace in the water bath for exactly one hour. (In high phosphatase sera, half quantities may be used or the incubation time reduced to one-half to one-fourth hour.)

reduced to one-half or one-fourth hour.)

At the end of the incubation period, remove from water bath

and cool immediately to below room temperature under running water.

Add 9 ml. of trichloroacetic acid solution, 10% to each tube, mix and let stand for about ten minutes.

Filter through a dry ashless filter paper (Whatman No. 44). This is the "total inorganic phosphorous" filtrate. Set aside for analysis. Analysis may be delayed for several hours without any error resulting.

Place 1 ml. of serum in a 15 ml. test tube. Add 4.5 ml. distilled water and 4.5 ml. of trichloroacetic acid solution, 10%. Mix by shaking, let stand for ten minutes and filter as above. This is the "serum inorganic phosphorous" filtrate.

Place 5 ml. of each filtrate into a clean marked test tube (18 x 150 mm.).

Place 5 ml. of phosphate standard solution, dilute in a similar tube.

To all tubes add 4 ml. of molybdic acid reagent, mix by tapping and then add 1 ml. of stannous chloride solution, dilute to each, mixing by a single inversion immediately. Let stand about three minutes, then read in colorimeter. Calculate results. (Readings may be made up to 2 hours without change in color. About twenty test tubes may conveniently be included in a series of analyses. Include two standard tubes and check before and after reading unknowns.)

Bodansky in a previous publication (16) explained that a correction should be made in the calculation to correct for the deviation from Beer's law and also for the effect of trichloroacetic acid plus glycerophosphate on the color intensity of the test. He reported that after a series of experiments along these lines he was able to establish a factor for each correction.

The correction for the deviation from Beer's law must be made first and directly to the aliquot of filtrate used in the test according to the formula:

$$\left\{ \frac{R_s \times 0.02 \times 1.2}{R_u} \right\} - 0.0040 = \text{mg. P in aliquot}$$

This result may be converted into mg. of inorganic P per 100 ml. by a second formula:

$$T \times \frac{100}{V} = \text{mg. P per 100 ml.}$$

T equals the corrected value of the aliquot and V the amount of serum used in the analysis (0.5 ml. in serum phosphate determinations and 0.25 ml. in serum phosphatase determinations).

The correction factors for the effect of trichloroacetic acid and glycerophosphate have been determined to be +1% per ml. of serum phosphorous filtrate used in the analysis (5% in the above method) and +2% per ml. of phosphatase filtrate (10% in the above method). These corrections are applied to the above results (mg. P per 100 ml. of serum).

Phosphatase units equal (mg. inorganic P of "total inorganic phosphorus" analysis, corrected) — (mg. inorganic P of "serum inorganic phosphorous" analysis, corrected).

1 unit Bodansky equals the equivalent of 1 mg. inorganic phosphate (expressed as P) liberated from sodium glycerophosphate substrate during the first hour at pH 8.6 and at 37° C., per 100 ml. of serum. Normal values—1.5 to 4.0 units (adults).

5.0 to 12.0 units (children).

King Method

King (9) is the most recent publisher of a method of phosphatase determination. The objects of his method are: To provide a more rapid as well as a more accurate method; to limit the total amount of serum for duplicate tests and controls to 2 ml.; and to express results in units numerically equal to those of Jenner and Kay.

He observed that the rate at which disodium-phenyl-phosphate is hydrolyzed by phosphatase was approximately double that for disodium beta-glycerophosphate. Theoretically, substitution of the former substrate should cut the incubation time in half or to one and a half hours. Also, the fact that phenol estimation gives the same measure of extent of hydrolysis as does the estimation of liberated phosphate and, in a given weight of phenylphosphate there is approximately three times as much phenol as phosphorous liberated, the hydrolysis time may also be cut further. Hydrolysis for thirty minutes will result in phenol liberated in amounts equal to that of phosphorous in one and a half hours.

Other features of this new method are: The discarding of Jenner and Kay's glycine-sodium hydroxide buffer in favor of Bodansky's veronal buffer in a greater concentration (pH remained more constant); adoption of a combined solution of buffer and substrate of excellent keeping qualities.

The phenol determination method of Folin and Ciocalteu was adopted to determine phenol liberated.

This method is based upon the hydrolysis of an excess of disodium phenyl-phosphate and the determination of the amount of phenol liberated. The phosphatase activity is calculated by differ-

ence between the phenol liberated in the test and the phenol in the serum control.

SOLUTIONS AND REAGENTS.

Buffered substrate solution. Dissolve 10.3 gm. sodium diethyl-barbiturate (veronal) and 1.09 gm. di-sodium phenyl-phosphate in distilled water in a 1 liter volumetric flask. Dilute to volume and mix. Preserve with 2 or 3 drops of chloroform in the refrigerator.

Phenol reagent of Folin and Ciocalteu. Dissolve 100 gm. of sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$) and 25 gm. of sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$) in 700 ml. of distilled water in a 1500 ml. Florence flask. Add 50 ml. of phosphoric acid, concentrated and 100 ml. of hydrochloric acid, concentrated. All reagents should be of cp. quality. Connect the flask with a reflux condenser by means of a cork or rubber stopper wrapped in tin foil. Boil for ten hours and then add 150 gm. of lithium sulphate, 50 ml. of distilled water and a few drops of liquid bromine. Boil the mixture without the condenser for fifteen or twenty minutes to remove the excess bromine. Cool, dilute to 1 liter, mix and filter. The finished reagent should have no greenish tint. Keep well protected from dust.

Phenol reagent, dilute. Dilute the above reagent 1 to 4. Prepare daily.

Sodium carbonate solution, 20%. Dissolve 200 gm. of sodium carbonate, anhydrous with the aid of heat, in sufficient distilled water to make 1 liter of solution.

Phenol standard solution, stock. Dissolve 1 gm. of crystalline phenol in hydrochloric acid N/10 in a 1 liter volumetric flask and dilute to volume with more acid. Mix well and titrate with iodine to determine actual strength.

To titrate, transfer 25 ml. of the phenol standard solution to a 250 ml. flask, add 25 ml. of iodine N/10 after adding 50 ml. of sodium hydroxide N/10 and heating to 65° C. Stopper flask and let stand at room temperature for thirty to forty minutes. Add 5 ml. of concentrated hydrochloric acid and titrate excess iodine with sodium thiosulphate N/10. Each ml. of iodine N/10 used (iodine added—sodium thiosulphate used in titration) corresponds to 1.567 mg. of phenol.

Phenol standard solution, dilute. Dilute a volume of the phenol standard solution, stock so that 1 ml. will contain 0.1 mg. of phenol.

Standard phenol solution-reagent mixture. Place 5 ml. of the phenol standard solution, dilute, in a 50 ml. volumetric flask. Add 15 ml. phenol reagent, dilute and make up to volume with distilled water. Mix thoroughly. 1 ml. contains 0.01 mg. of phenol. Prepare daily.

METHOD.

Prepare serum by centrifuging twice.

Place 10 ml. of buffered substrate solution in each of two test tubes.

Place in a water bath at 37° C. for about five minutes.

Add 0.5 ml. of serum to each tube, stopper, mix by inversion and place in water bath again for exactly thirty minutes.

Just before incubation time is complete, set up two control tubes containing 10 ml. of buffered substrate solution and 0.5 ml. of serum in each.

At the end of the thirty minute incubation period, remove the tubes and cool rapidly under running water.

To all tubes add 4.5 ml. phenol reagent, dilute, mix thoroughly and filter through a dry ashless filter paper (Whatman No. 44—9 cm.).

Pipette 10 ml. of each filtrate into a clean marked test tube.

Pipette 10 ml. of standard phenol solution—reagent mixture into a similar tube.

To all tubes add 2.5 ml. of sodium carbonate solution, 20%. Mix and place in water bath for five minutes to bring out full color. Read in colorimeter and calculate according to the formula:

$$\frac{Rs}{Ru} \times 0.1 \times 1 \times \frac{100}{0.33} = \text{mg. phenol per 100 ml. serum.}$$

$$\text{or condensed } \frac{Rs}{Ru} \times 30 = \text{mg. phenol per 100 ml. serum.}$$

Phosphatase units equal (mg. phenol per 100 ml. of serum in phosphatase analysis) — (mg. phenol per 100 ml. of serum in control).

1 unit King equals the equivalent of 1 mg. of phenol liberated from an excess of disodium phenyl-phosphate in thirty minutes at 37° C. and at pH 9.0, per 100 ml. of serum. Normal values—3.0 to 14.0 units.

Comparison of Methods

We have run a series of analyses in an attempt to make a comparison between the three methods just described. Our findings are quite conclusive.

In general, the three methods compare as follows: The Jenner-Kay method is, at the very best, with its glycine-sodium hydroxide

buffer and long incubation time, a very cumbersome and time consuming one. In our experience, we find it the least accurate of the three. King's method is complete and short, but we feel that errors are apt to arise due to the great variation between the color density in the standard and the control tubes. No difficulty is encountered in reading the phosphatase determination against the standard set at 15 or 20 mm. However, in order to read the control the standard must be set at 5 mm. or less. This is obvious when the amount of serum used in the control (0.33 ml.) is considered. We believe that this is the one defect of the test, and, that it may be remedied by increasing the amount of serum used in the control. We hope to be able to report a modification of the method to eliminate this defect.

The Bodansky method, we believe, is the most preferable. It is based upon the greatest amount of experimental work and corrections are made for the deviations from Beer's law and the influence of trichloroacetic acid and glycerophosphate on the color density. Neither of the other two authors take these errors into account. Bodansky claims the limit of error to be less than $\pm 2\%$. In our hands, this method was very effective and not too time consuming. In fact, after due consideration, we decided to base our comparison on this method.

To compare the methods from the unit basis, theoretically the Jenner and Kay unit is equal to the King unit, and both of these are equal to three Bodansky units. Considering the normal values reported for each (Jenner-Kay 3.2-7.9, Bodansky 1.5-4.0, King 3.0-14.0) we find that the above relationship does not exist. Instead the Jenner-Kay unit is equal to two Bodansky units and to about two-thirds of a King unit. Experimentally we found this last relationship more common than the first.

Comparing our results with Bodansky's method as a basis, we found very close agreement between his method and King's according to the above theoretical expectations (within a ± 5 units or 10%). The Jenner-Kay method showed a very large error ($\pm 40\%$) over the theoretical expectation, but a more closer agreement ($\pm 15\%$) on the basis of 1 unit equals two Bodansky units. Our results with King's method as a basis, showed an error of a $\pm 35\%$ between it and the Jenner-Kay method according to the theoretical expectation; and an error of a $\pm 20\%$ on the basis of 1 unit Jenner-Kay equals two-thirds King unit.

Our results agreed with the findings of all workers, of which Bodansky's findings are representative. A table of Bodansky's results in various pathological conditons follows:

Polyostotic Pagets disease	50.0—135.0 units
“ “ “ with healing	15.0— 50.0 “
Localized “ “	5.0— 20.0 “
Osteosclerosis fragilis	15.5— 21.3 “
(Marble bones, Abers-Schonberg disease)	
Infantile rickets	30.0—190.0 “
Healed “	6.0— 14.0 “
Multiple Myeloma	1.8— 28.1 “
Generalized osteoporosis	5.0— 10.0 “
(up to middle age)	
Senile osteoporosis	1.5— 4.0 “
Clinical hyperparathyroidism	about 25.0 “
Osteomalacia	High
Hyperthyroidism with bone changes	High normal to slight increase
Erythroblastic anemia	“ “ “ “
Osteomyelitis	“ “ “ “
Fractures	“ “ “ “
Gaucher's disease	“ “ “ “
Arthritis	High
Bone cysts	“
Acromegaly	“
Fragilitus osseum	“
Non-bone cysts	“
Achondroplasia	“
Osteogenesis imperfecta	“
Obstructive jaundice	High
Other types of jaundice	Normal

We wish also to call attention to the improved method of inorganic phosphorous determination as used by both Jenner and Kay and Bodansky. It is a modification of the Kuttner-Lichtenstein method (17) reported by them in 1930.

Summary

1. Three methods of phosphatase determinations are reported in detail.
2. A comparison of the three methods is made based upon results found.
3. Attention is called to an improved method of inorganic phosphorous determination.
4. A table of results (Bodansky) in various pathological conditions is included.

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STATISTICAL STUDY OF ICTERUS INDEX IN VARIOUS CONDITIONS

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This is the means by which the depth of color of the serum may be expressed by a number. So far as is known, the yellow color of the normal blood serum in the fasting person is due to bilirubin. The color of the serum is made deeper color by an increase in bilirubin. This occurs in:

- (1) Hemolytic processes, such as pernicious anemia; hemolytic jaundice; hematoma; rupture of a viscus; and malaria.
- (2) Disturbances of the biliary system, such as cholangitis; cholecystitis; cholelithiasis; adhesions about the gall-bladder and diseases of the liver.

A serum of paler than normal has been observed in all cases of secondary anemia. As normal bilirubinemia depends on the normal destruction of red blood cells, it is conceivable that the pale serum in secondary anemia is due to reduced number of red cells in this condition, with a consequent diminished amount of bilirubin.

The bilirubin content of the blood may be considerably increased above the normal before icterus is perceptible in the sclerae or other tissue. Obviously, a test for the detection of this so-called latent jaundice is of considerable value, especially in those conditions in which diagnosis is doubtful.

Technique

Several c.c. of blood are withdrawn from the vein, allowed to clot and then centrifuged. The supernatant serum is removed with a pipette (2 c.c.) and diluted with N. saline and compared with an arbitrary standard of 1-10,000 potassium dichromate. The color is compared with a colorimeter taking 15 as the standard.

Calculation

The standard number 15 is then divided by the scale reading of the unknown. The quotient is the icterus index. If the scale reads 3 on the unknown then the index will be 5. However, if the color of the serum is too deep and it is necessary to dilute with normal saline, then the quotient is multiplied by the number of dilutions. If the dilutions were 10, then the index would be 50.

The deeper the color of the index serum, the higher the index. The normal range has been found to be between 4-6. The lowest

reading was 2.3 and the highest was 282 and was found in a case of yellow atrophy of the liver due to cinchophen.

Errors in Technique

The standard solution becomes paler on exposure to light. Solution may be made more stable by adding 2 drops of sulphuric acid to 500 c.c. of the potassium dichromate. The serum must be clear—cloudy or lipemic sera give false readings.

Readings must be less than 24 hours standing, as the serum becomes opaque on long standing.

Hemolyzed serum must be discarded.

Needles and tubes must be dry on drawing blood.

Avoid transferring blood from one tube to another and it is advisable to collect the blood in the tube that goes in the centrifuge.

There are substances in the serum that deepen the color other than bilirubin. In infants, after the ingestion of eggs, oranges and chlorophyll, containing vegetable carotin and xanthophyll are the substances contained in these foods that produce the same effect. Eating of carrots by an adult will cause a high index two hours after. Will return normal on next day. It is conceivable that the power of absorbing coloring matter which may effect the color of the serum may differ with different individuals. Taking the index in the fasting state, will obviously rule out the possible influence of such substances.

Clinical icterus has invariably been present when the index was above 15 and invariably absent when the index was below 15. The zone of latent jaundice may accordingly be said to lie between 6 and 16. In tests made, in some persons, the readings on repetition was constant except during periods of digestion, when the serum is cloudy and cannot be read with accuracy.

The Anemias (109 Cases)

The index ranged from 6.5 to 12.5 in pernicious anemia. In secondary anemia, the index varied from 2.3 to 3.9. In no other series of cases was the index so low. In carcinoma of stomach, such was noted. The anemia accompanying this condition has been thought by some to be due to hemolysis of the red blood cells produced by the toxins of the disease. The low icterus index findings indicating a decrease in bilirubin show that this anemia is not of a hemolytic character.

Gastric Ulcers (124 Cases)

The index findings were of normal figures.

Duodenal Ulcers

The index findings ranged from 7.5 to 13.6. Possibly the high

index here is accounted for by adhesions about the gall-bladder or an extending duodenitis involving the papilla of Vater with a cholangitis.

Gall-Bladder Disease (116 Cases)

The index is perhaps of greatest value in doubtful conditions of the right upper quadrant. The surgeon often has difficulty in determining whether disease is due to gall-bladder, the kidney or appendix. In disease of the gall-bladder in which there is no clinical jaundice, but which is sufficiently severe to produce symptoms, the blood will show an increase in bilirubin, owing to the interference with the biliary system. A high icterus index, therefore, indicates involvement of the gall-bladder, while in kidney disease and appendicitis, the index is normal. However, accidental findings of gall-stones at autopsy give evidence that cholelithiasis may exist without noticeable disturbance of the biliary system. There is no reason in such cases to expect an icterus index above the normal. Indices varied from 7.1 to 15 in gall-bladder disease. In every case after operation, the index returned to normal.

Clinical Jaundice (112 Cases)

The index is of value not only in the zone of latent jaundice, but also in cases of frank clinical jaundice in which it is not possible from day to day by observation alone to determine whether the jaundice is increasing or decreasing.

Malaria and Typhoid (92 Cases)

7.5 to 8 in Malaria.

9 to 11.5 in Typhoid.

In malaria, the high index is evidently due to destruction of red blood cells, caused by the parasites in the disease, whereas in typhoid, the biliary obstruction is probably the causative factor; typhoid bacilli may be in the bile and also a fecal necrosis in the liver. The index is higher in biliary disease than in hemolytic conditions.

Trichinosis (47 Cases)

The index was normal; may get a positive Widal in this disease, but the high icterus index in typhoid is a differential point.

Cardiac Disease (114 Cases)

A subicteric color of the skin has been commonly observed in many cases of cardiac insufficiency especially in those showing passive congestion of the liver. This color is due to a hyperbilirubinemia, the measurement of which by means of the Icterus Index appears to be of some prognostic value. In observations made of myocardial and chronic cardiac valvular decompensation, the degree of index varied with the amount of decompensation. In cases with

no decompensation, the Icterus Index was normal. 7 to 9 index in mild cardiac failure. 12.5 to 15 in severe or fatal cases.

Pneumonia (64 Cases)

Frank jaundice in this disease is a sign of poor prognosis. This index may show a latent jaundice, and therefore of value in early prognosis. No case in which 7.5 is the index terminates fatally. In some cases, where there is a secondary anemia-complication, the index would be low.

Group III Pneumococcus (highest mortality) showed the highest index. Those cases of Group III Pneumococcus infection showing a low index recovered.

Diabetes Mellitus (260 Cases)

Index ranged from 7.5 to 15. The explanation of these figures is not clear. There appears to be a close connection between the sugar and pigmentation metabolisms. In a diabetic patient (experiment), the index was high while in fasting state, becoming lower with rise in blood sugar. In normal persons, there was a normal index while fasting and higher index as the blood sugar rose.

Other Findings (42 Cases)

The highest indices were obtained in cases of occlusion of the common bile duct, in catarrhal jaundice and carcinoma of liver, 2 cases of ectopic pregnancy with rupture and intraperitoneal hemorrhages had indices of 9 and 15.

Fractures of the pelvis, tibia and femur gave indices of 9 and 11 (for 24 hours); hematoma of the popliteal space gave an index of 10.

Summary

1. There is a normal bilirubinemia. There may be a hypo or hyper-bilirubinemia.
2. Determination of the Icterus Index is an accurate estimation of the degree of bilirubinemia.
3. The Icterus Index as a measure of latent jaundice is an aid in diagnosis, and prognosis in a number of diseases.
 - (a) It differentiates the hemolytic from non-hemolytic anemias.
 - (b) It shows that anemia due to carcinoma is non-hemolytic in character.
 - (c) It has been found to be normal in cases of gastric ulcer and high in cases of duodenal ulcers.

- (d) In doubtful abdominal lesions, it is of value in indicating biliary obstruction.
- (e) It shows whether jaundice is static, increasing or decreasing.
- (f) It has been found to be high in malaria but higher in typhoid.
- (g) It is normal in trichiniasis in which a positive Widal reaction is sometimes found. Therefore a differential between these two diseases.
- (h) It is of prognostic value in cardiac disease.
- (i) It is of some prognostic value in pneumonia.

4. The results of sugar tolerance tests performed on diabetic patients and normal persons suggest an interesting relationship between the sugar and pigment mechanisms which seem that further investigations would be necessary and worthy.

5. Sources of error in the performance of the test are readily controlled.

THE A. S. C. L. T. QUESTIONNAIRE

3. BACTERIOLOGY, SEROLOGY, PATHOLOGY, AND MISCELLANEOUS

By SISTER M. JOAN OF ARC WILSON, R.S.M., and
CHARLES E. BRAMBEL, PH.D.

From the Department of Laboratories, Mercy Hospital, and the Department of Zoology, Johns Hopkins University, Baltimore, Md.

In our third series we have condensed into one unit all the information received from the questionnaire papers on Bacteriology, Serology, Tissue Pathology, Basal Metabolism, Electro-cardiography, Pregnancy Tests, and Intravenous Solutions. The data submitted were so heterogeneous that no detailed summary is possible in the space available.

The returns from the Bacteriology section showed the most varied choice of methods of any department of laboratory work. The selection of culture media in the different laboratories was so voluminous as to prohibit enumeration: we therefore selected only the most popular combinations for our graph, and included all the rest in the miscellaneous section, representing 27%, and including about forty different admixtures of media.

As indicated in Figure 1, 54% use some form of both agar and broth as routine media for general culture work, 6% use broth only, and 6% agar only. The agar includes plain, blood, chocolate blood, brain veal, dextrose, ascitic, Kracke's, Sabouraud, Loeffler's, and other combinations. The broths include beef infusion, nutrient, dextrose, bacto brain veal, beef heart hormone, ox-bile, cabbage nutrient, and other varieties.

In the selective medias for gonococcus, B. pertussis, and pneumococcus, there was a definite choice for enrichment with blood, either in agar, broth, or on potato: however, the ascitic or hydrocele fluid media predominated for Gonococcus in the 39% of the laboratories which reported culturing for this organism. For diagnosis of pertussis, 28% prefer the cough plate method, and 14% gave other miscellaneous procedures.

A large majority use some form of broth and additional agar plates for culturing the blood either as routine or in specific fevers, such as typhoid, pneumonia, and general septicemia. Nine per cent reported collecting the blood first in citrate, and 1% use the Keidel

tube. For the typhoid bacillus, only 19% reported using bile broth media, and 6% endo agar, while a still smaller group use the eosin-methylene-blue agar. Kracke's agar or broth is preferred by approximately 5% as a blood culture media, and Cecil's and Clawson's technics were included in the special methods. Only 43% gave any data on the percentage of positive cultures in definite septicemias and how the results checked with the progress of the disease. Of this number the positive cultures ranged from 3% to 100%, the minimum percentage increasing with the increase of bed capacity of the institution.

For the general examination of sputa, 10% gave both macro- and microscopic procedures, 10% cultured the sputa routinely, 47% reported making a Gram stain, 79% an acid fast stain for tubercle bacilli, of which 76% use Ziehl-Neelson technic; and 17% gave various other determinations. For a more detailed search for tubercle bacilli, 52% reported animal inoculations, and 25% use various concentration methods. Corper's potato media is used by the majority who culture for this organism. Where both animal inoculations and cultures are made simultaneously, 11% reported an excellent check in their results, 35% good results, and 6% only fair results.

Additional animal inoculations reported by only a few institutions are as follows: guinea-pigs for glanders, undulant fever, diphtheria, staphylococcus or streptococcus, *B. pestis*, jaundice; mice or rats for the pathogenicity of monilia; rabbits for Welch's bacillus and dysentery; cats for belladonna poisoning. One institution reported as follows: "Through blood culture of a Malta fever patient we recovered an organism and upon injection into a guinea-pig it seemed to have no effect upon the pig physically, but the animal's blood agglutinated the organism of the porcine type."

It appears that a great variety of procedures are utilized in the laboratory work on typhoids. As routine, only 69% make the Widal test, 2% a urine diazo, 0.2% blood typing for a possible hemorrhage emergency. The cultures made routinely are as follows: blood, 87%; feces, 51%; and urine, 37%. In differentiating typhoid bacilli from the other intestinal organisms, 45% prefer the endo media, 30% Russell's sugar media, 16% eosin-methylene-blue agar, and 14% other selective medias.

The bile solubility test holds the precedence of 30% in differentiating pneumococci from streptococci, though the Hiss-Inulin-Serum-Water media is used by 12%. Blood agar was also listed as a differentiating media, and there were forty-four other combinations of media or agglutination procedures enumerated.

Only 10% of the institutions (the majority being in the group of 100-200 beds) reported making routine nose and throat cultures for *B. diphtheriae* on children admitted to the hospital: the positives ranged from 0.1% to 95%. Also, only 10% reported making smears for gonococci preliminary to admission of little girls; the positives ranged from 0.01% to 10%.

The preparation of autogenous vaccines was reported by 58%, the Hopkins tube being used by the greater number. Cohen's Pathogen-Selective method was reported by 0.7%; the others merely stated saline suspension or heat sterilization methods; and fifteen other procedures were listed. Therapeutically, the saline suspensions are more preferred than the bouillon filtrates or the sensitized vaccines, and 12% gave combinations of the preceding. The skin tests for sensitivity are made by 51%.

Where vaccines are administered by the laboratory, the results are as follows:

	Not done	Excellent	Good	Fair	Poor
Staphylococcus Infection	85 %	2 %	9 %	3 %	—
Intestinal Disorders	91 %	1 %	2 %	3 %	3 %
Chronic Respiratory Infection	90 %	0.2 %	4 %	4 %	1.8 %
Arthritis	91 %	1 %	3 %	3 %	3 %

Figure 2 depicts the returns from the Serology section and the other departments considered in this series.

As the majority of the institutions use more than one test routinely for the diagnosis of lues, we have classified the percentages according to the total number using the various methods, and also the number using two different tests simultaneously, specifying the reported checks on the two methods. The Kahn precipitation test reached the highest percentage (61%) of any one specific test; the flocculation methods reached 77%, and complement fixation 75%. The percentage of agreement between complement fixation and Kahn was only 55, and between Kahn and Kline, 20.

At the time of this survey the reports indicated that the mouse inoculation for typing pneumococci was still used by a majority of 31%; however, Neufeld followed closely with 28%, while Krumweide was only 14%. The remaining institutions did not give any data on this question. Of those who inoculate mice, 26% follow up with the macro-agglutination test, 23% with the macro-precipitation, and 18% utilize the Sabin slide method. In determining the type incidence of pneumococci, 31% use Types 1, 11, and 111 antisera, and 1% use all the types from 1 to XXX111. Besides these

SEROLOGY - PATHOLOGY AND MISCELLANEOUS

ROUTINE DIAGNOSIS		WASSERMANN COMPLETE REACTION		KINSE	
# SYPHILIS		KINSE		KINSE	
CHECK 4 RESULTS MORE THAN ONE USED		KINSE		KINSE	
NON REAGENTS AND ANTIGENS RECAPTURED		KINSE		KINSE	
TYPING PRENUCOCCL		KINSE		KINSE	
TYPE ANT SERA USUP		KINSE		KINSE	
PRENUCOCCL TYPING		KINSE		KINSE	
UNDULANT FEVER		KINSE		KINSE	
TULAREMIA		KINSE		KINSE	
TYPHUS FEVER		KINSE		KINSE	
TYPHOID FEVER		KINSE		KINSE	
RHEUMATOID ARTHRITIS		KINSE		KINSE	
HISTOLOGICAL		KINSE		KINSE	
PREVAILING		KINSE		KINSE	
DIAGNOSIS		KINSE		KINSE	
B. M. R.		KINSE		KINSE	
GLUCOSE CARDIO-		KINSE		KINSE	
GLUCOSE		KINSE		KINSE	
HYPERGLUCOSE		KINSE		KINSE	
HYPERGLUCOSE		KINSE		KINSE	

Figure 2

figures, there were numerous other combinations of antisera used.

An average of 20% of the hospitals call upon their local health departments for the bacterial agglutination tests in specific fevers, such as typhoid, tularemia, typhus, and Malta fever; though 65% reported making their own agglutination tests for typhoid, and an average of 18% gave various methods for determining the etiology of the other fevers. The macro-agglutination test was the most utilized procedure where several were given.

Of the methods used for histological sections, the paraffin predominated with 55%; celloidin only 8%. Though 42% reported frozen sections, these were mostly in conjunction with one of the other procedures, and a few gave isolated miscellaneous technics.

The increasing popularity of the pregnancy tests was demonstrated by the data received from 69% of the institutions which showed that the Friedman rabbit test was used by an excess of 10% over the Aschheim-Zondek method, and the positive findings proved to be 90%-100% correct in both methods. X-Ray and Mazer-Hoffman methods were also listed.

Ten different basal metabolism machines were reported as being used, and of those who gave data on this question, 42% bring the patient to the machine, and 28% take the machine to the patient, while 20% reported both methods. Although the laboratory technician is responsible for the metabolism tests in the majority of the institutions, 79% stated that the electro-cardiographic unit was not under the supervision of the clinical laboratory.

As there has been so much controversy about the preparation of intravenous glucose and salt solution, inquiry was made as to where these were prepared and also the technics used. The replies were quite striking in that the laboratory was responsible in less than 4% of the institutions for their preparation; 4% purchase the glucose in ampoules, and in the balance either the operating room, pharmacy, or central supply department prepare these solutions. Of those who gave technic, practically all use Merck's C. P. sodium chloride and anhydrous dextrose. The sterilization was reported for varying lengths of time, from 20 minutes to 1 hour; and the pressure, from 0 to 15 pounds.

Erratum

Bulletin No. 6, Vol. 1, Nov. 1935, page 189. Line 28 reads:
"It was interesting that only 97% reported any incidence of —"
and should read:

"It was interesting that only 9.1 % reported any incidence of —"

EDITORIALS

ON THE WRITING OF MEDICAL PAPERS

"Of the making of books, there is no end" once said a learned and wise observer, but even he did not know that his truism could correctly be applied to medical papers. Medical literature has now become so vast that almost any subject, however minor, is treated in so many articles that one must indeed have tireless eyes to but scan the published documents.

Even a casual study of much, if not most of this literature, will reveal errors of fact, thought, logic, form or language and often more than one of these which leads to the conclusion that few who submit manuscripts for articles, monographs or books have had adequate training in writing. To be sure, if the facts are incorrect it makes no difference how perfect the logic, form and language are, and this leads to the first premise that one must not only have something to write about but the facts must be correct. This concept is wrapped up in network made up of such fundamental threads as intelligence quotient, training, experience, ability to observe, accuracy, patience, honesty, integrity, and a host of other qualities and virtues. After the facts are assembled, one must plan carefully the contents of the proposed article. Either a mental or a visual outline should be made. This should be as elaborate as the subject demands and it is essential, in order that the subject matter may be presented logically, sequentially, and without repetition. It will serve to prevent undue lengthiness, a feature all too evident in many medical papers.

When the writing has actually commenced, the author should state his viewpoint and stick to it, or if, as the subject is unfolded, it becomes necessary to change the viewpoint, the reader should be "let in" on the secret.

Two or three books should always be at hand as the article is written and rewritten and should include an English dictionary, a medical dictionary, and an elementary grammar. No one ever becomes so experienced that these can be omitted from consideration and consultation. Frequently a calendar, atlas, and ruler will be essential to prevent ridiculous errors which sometimes appear in texts.

Just what to include in the manuscript, which of course is to be typed and double spaced, has to be settled by the author and an

editor but, in general, long protocols either in the form of text or tables should be omitted. The author should be able to provide them when experts in the field desire to obtain some specific point but, generally speaking, they are so much wasted space in a Journal. If tables are used they should be clear without the necessity of guessing their meaning and the figures in them should match those in the text. If one's arithmetic is poor, he should use a machine to obtain the results or ask a mathematically minded friend to verify his addition and subtraction. It is not too much to expect a proper statistical treatment of data which lend themselves to such devices. Illustrations should be reduced to the essential ones and if photomicrographs are submitted they should be trimmed to show only enough of the structure necessary for an elucidation of the lesion or the structure. Prints must be sharp, adequate for proper reduction, and printed on glossy paper.

All references to literature should be correctly documented and credit to previous authors should be punctiliously given. If one differs from another author, the difference should be clearly set forth in dignified and courteous language. The most effective method is often to state the facts without comment.

The conclusions, every reader has a right to expect, should not fall outside the presentation of the material and should be limited to the matter given in the body of the text. They should be stated in simple direct language.

Finally, when the editor makes some much needed corrections in form and language, the author should take a philosophical attitude and realize that an editor of a medical journal is never satisfied.

THOMAS B. MAGATH.

PAST AND PRESENT POLICY

The public at large does not place the laboratory technician in the same category as in years gone by. Time, alone, has been a great factor in drawing attention to this type of worker; continually pointing to ability that is so evident in undivided attention to detail and coupled with dexterity, that is apparent only when the trained mind comes into action.

Through the medium of the Board of Registry of the American Society of Clinical Pathologists the status of all qualified laboratory technicians has been raised. At the expense of those who are registered, those who are qualified and have not registered are no doubt reaping where they have not sowed. The American Society of Clinical Laboratory Technicians is composed only of laboratory

technicians who are registered and fosters a unity of purpose in a worth while vocation.

The "American Journal of Medical Technology," the official organ of the American Society of Clinical Laboratory Technicians, endeavors to publish only articles that are beneficial from the laboratory technicians' viewpoint. The technician who submits articles to be published, with this in mind, does in no way digress from the code of ethics which he solemnly avows to uphold.

Where is there a laboratory director who does not wish for a loyal, intelligent staff of technicians? The individual who evolves a new method or simplifies old lengthy procedures, or is able to suggest some theories on laboratory procedures hitherto unclarified, is worthy, from the point of right, and may, even while holding true to the code of ethics, publish such findings. It is possible that there may be some who would attempt to suppress the efforts of technicians along these lines. Such tactics are not regarded favorably by the American Society of Clinical Laboratory Technicians. The Society shall remain strictly ethical, and shall employ the Journal for the benefit of all registered laboratory technicians and shall furnish them a means of publication.

NEWS AND ANNOUNCEMENTS

BOARD OF REGISTRY

OF A. S. C. P.

Who May Teach Student Technicians?

In the September issue of the "Bulletin" a warning was sent out by the Registry against unsupervised and independent training of students by Laboratory Technicians. Pressure is occasionally exerted by Superintendents, Trustees, or Members of the Medical Staff to impart instruction to aspirants for laboratory training. Often these students have an inadequate preliminary education or the request is for a short course of instruction. The practice is, of course, pernicious and not at all conducive to elevation of the status of this calling.

In our zeal to condemn this harmful procedure, we overlooked the possibility of misinterpretation of this ruling on the part of technicians working under the latter's supervision. We, therefore, desire to reassure these conscientious technicians that they may continue, as heretofore, to assist the director in the teaching of students.

It is manifestly impossible for the clinical pathologists to do all the teaching in person. He must, and for obvious reasons does, delegate this work to the respective heads of the various departments of the laboratory or to competent assistants but always under his immediate supervision.

We have no doubt that the above explanation will clarify the matter and remove all doubt as to who may exercise this important pedagogical function.

Four hundred and twenty-four applicants appeared before the one hundred and seven examiners for the October, 1935, tests, which were held in various cities in the United States and Canada.

The following questions were used in the Written Examination of Applicants for Registration as Laboratory Technicians:

1. Describe briefly and systematically the nature of laboratory work in which the applicant is engaged; name the divisions of the laboratory in question, and the general set-up of the laboratory.
2. Name 4 species of laboratory animals commonly used in the

clinical laboratory. Name 1 important use for each species. Name 5 routes used in animal inoculation.

3. What is the difference between a vaccine and an anti-serum? What type of immunity does each produce?
4. What is meant by hydrogen ion concentration?
5. By what method is blood creatinin usually determined?
6. (a) Describe technic involved in staining by Gram method. Indicate "Gram positive" or "Gram negative" for each of the following:
 - B. coli (*Escherichia coli*).
 - M. gonorrheae (*Neisseria gonorrheae*).
 - B. influenzae (Pfeiffer) (*Hemophilus influenzae*).
 - Staphylococcus aureus*.
- (b) Describe a method of preparing suspected tuberculous sputum for guinea pig inoculation and for culturing.
7. Describe three methods of determining the hemoglobin content of blood. State choice of hemoglominometers.
8. What is meant by (a) complement fixation test? (b) flocculation test? Give examples of each.
9. In what manner does the technic differ when temporary frozen sections and permanent frozen sections of tissue are prepared?
10. How may the urea content of urine be determined quantitatively?

An Exhibit of the Registry was held at the Southern Medical Meeting, at St. Louis, by Doctor Roy R. Kracke, in November.

NATIONAL

COMMITTEES—1935-36

Program

Frieda H. Claussen, M.T., 469 Laurel Ave., St. Paul, Minn., chairman.

Vivian Herrick, L.T., 4929 Lake Park Ave., Chicago, Ill.

Luella Gifford, M.T., 339 Boush St., Norfolk, Va.

Frieda Ward, L.T., Hospital of St. Barnabas, 685 High St., Newark, N. J.

Myrtle L. Sand, L.T., 1850 W. Jackson Blvd., Chicago, Ill.

The Program Committee is desirous of receiving applications from the members of the Society who wish to appear on the program of our Annual Session in Kansas City, next May.

They are requested to communicate with Miss Frieda Claussen, M.T., chairman program committee, 469 Laurel avenue, St. Paul, Minn., as early as possible and not later than February 15, 1936, stating the title and giving a brief summary of the paper to be presented.

It is also proposed that we conduct a "Round Table Discussion," as one of the features of the program. The members are invited to send in written questions on daily problems and procedures on which they wish to be enlightened, not later than April 1, 1936. The Committee will endeavor to place these questions in the hands of those best qualified to answer them, and have them discussed at one of the sessions.

The success of our program depends entirely upon the response the Committee receives from the members, whose co-operation is earnestly solicited.

Scientific Exhibits

Paul C. Brown, M.T., Newark Beth Israel Hospital, Newark, N. J., chairman.

M. Elisabeth Cramer, L.T., 164 Market St., Lexington, Ky.

Myra C. Effinger, M.T., Altoona Hospital, Altoona, Pa.

Bernice Elliott, L.T., 5107 Webster St., Omaha, Neb.

Arthur T. Brice, Jr., M.T., 144 Twenty-seventh Ave., San Francisco, Cal.

Cecil H. Gowen, M.T., 1 Madison St., Glenridge, N. J. (Ex-Officio).

Charles Brambel, Ph.D., Johns Hopkins University, Baltimore, Md. (Ex-Officio).

An interesting feature of the convention to be held in Kansas City, Mo., May 11-12-13, will be the Scientific Exhibits. All members wishing to participate are requested to get in touch with the nearest member of the Exhibits Committee, or to write directly to the chairman, Mr. Paul C. Brown, M.T., Newark Beth Israel Hospital, Newark, N. J., as soon as possible, and not later than February 15, 1936.

Research

Phyllis Stanley, Newark, N. J., chairman.

Faith Dravis, Minneapolis, Minn.

Henrietta Lyle, Columbia, Pa.

Paul Mader, Los Angeles, Cal.

Frances de Vaux, Birmingham, Ala.

Dr. C. E. Brambel, Baltimore, Md. (Ex-Officio).

Sister M. Joan of Arc, Baltimore, Md. (Ex-Officio).

The Research Committee as provided for in the Constitution has just been appointed. One piece of Society research in the form of a questionnaire has been presented by Sister M. Joan of Arc and her co-workers. This year the committee hopes to be of service to members, and can be in proportion to the response it receives. By convention time it is hoped that a cross catalogued file of contribu-

tions to the literature made by our members will be available. Reprints, whenever obtainable, will be filed for use. The committee will attempt to establish an information bureau as a source of references to laboratory procedures and such data as members may request. Such information should be useful to all, but especially to members who do not have access to libraries. Address inquiries to any member of the committee. A question blank is enclosed with this Journal. Please fill it out and return as soon as possible.

We hope to have the Committee on Entertainment and Local Arrangements ready in time for publication in the March issue of the Journal.

The Editorial Office has been informed that the first international meeting on fever therapy will be held in New York City, September, 1936. The use of fever induced by physical and other agencies as a therapeutic procedure has received universal attention in the past few years. The conference will aim to collect and crystallize available data in this field. Therapeutic, physiological and pathological phases of fever will be discussed.

Further information concerning the conference may be obtained from the Secretary. Baron Henri de Rothschild, Chairman, Paris, France. Dr. William Bierman, Secretary, 471 Park Avenue, New York City, U. S. A.

Dr. John A. Kolmer, Professor of Medicine at Temple University in Philadelphia, Pa., was awarded the Ward-Burdick medal for his work on poliomyelitis vaccine. In recognition and appreciation of this contribution and his other achievements in medical science, the LaSalle College, on June 6th, conferred upon him the honorary degree of Doctor of Science; and St. Joseph's College, on June 11th, the honorary degree of Doctor of Humane Letters.

At the annual convention of the A. S. C. P. the gold medal for scientific exhibits was awarded to Dr. Russell Haden, and the silver medal to Dr. Anna M. Young and her associates. Honorable mention was given to Dr. E. von Haam.

STATE

Arkansas

The Arkansas Society of Registered Laboratory Technicians has been organized. The first meeting was held on November 5th, 1935, during which the following officers were elected: President, Miss Ann Snow; Vice-President, Mrs. Clark; Secretary, Miss Amelia Metrailler; Treasurer, Miss Juanita Straubie.

Subsequent meetings will be held on the second Tuesday of each month.

Illinois

In May, 1932, a society was organized in Illinois which, up to the present time, had been meeting informally twice a year. At a meeting held in Urbana on October 19, 1935, through the instigation of Mr. Robert C. Jenkins of Chicago, a constitution was formally adopted, and steps taken to arrange for the affiliation of the Illinois Society with the A. S. C. L. T. The officers are as follows: H. M. Chiles, president; Ward Cade, vice-president; Exxa Bennett, secretary-treasury.

Through the efforts of the program chairman, Fanny B. Warnock, the following most successful program was carried out:

Friday, October 18—Registration and room assignment, Laboratory of Clinical Pathology, Burnham City Hospital, Champaign, Ill. 6 p. m., dinner at Winter's Tea Room. Opening Meeting—Dining room, Mercy Hospital, Urbana; presiding, Margaret Burgess. President's Greeting, Our State Organization, H. M. Chiles. Standards for the Laboratory Technician, B. Markowitz. Studies on Pasteurization of Milk in Relation to Undulant Fever, J. P. Torrey. Studies on Rabies Examinations in Past Year, with Moving Pictures, Viola M. Michael.

Saturday, October 19—Class Room, Burnham City Hospital; presiding, Fanny B. Warnock. 9 a. m., Demonstrations: Direct Nesslerization for Blood Urea Determinations, H. M. Chiles; A Serum Diagnostic Help in Cancer, Ward Cade; Rapid Method of Typing Pneumococci, S. E. Park. 10 a. m., Report from National Convention, Gladys Eckfeldt; Business Meeting: Presentation of Proposed Constitution, and Discussion led by Robert C. Jenkins. 11 a. m., Recess—followed by visit to Laboratory of Physiological Chemistry and Bacteriology at University of Illinois. 1 p. m., Luncheon. 2 p. m., Chemistry Building, University of Illinois; presiding, Ward Cade. Unfinished Business. Demonstration of Bacteriophage, B. E. Gay. Blood Uric Acid, H. E. Carter. Studies on Galactose Tolerance Test, Mrs. Beatrice Siemon. The Gram Stain, F. M. Clark. 4 p. m., Visit to Laboratory of Animal Pathology. 6:30 p. m., Dinner; Round Table discussion of topics requested but not already covered in the program: Reliable Methods for Determining Hemoglobin; Best Methods and Stains for Blood Films; Differentiation of Cells in Cerebrospinal Fluid; Classifying Blood Cells; Brucella Abortus Infection; Importance of Sulphur Determinations in Finger Nails; A Simple Method for CO₂ in Blood; Practicability of Filing Under Diagnosis.

Sunday, 9:45 a. m.—Visit to Clinical Pathological Laboratories of the Twin Cities for those who desired to remain over Saturday night.

Maryland

The November meeting of the Maryland Society of Registered Technicians was held at Mercy Hospital, Baltimore, at 8:15 p. m., in the laboratory amphitheatre. The following very interesting papers, which were supplemented by lantern slides, were given by doctors from Johns Hopkins Medical School:

"Technical Methods for the Differentiation of Streptococci"—Dr. J. Howard Brown. "Assay of Adrenal Cortical Hormone"—Dr. W. M. Firor.

Following the papers there was a general discussion of technical procedures of various kinds.

Among the large attendance were a number of members from the District of Columbia Society, representatives from the City Health Department, also from University of Maryland Medical School, and the student body of Mercy Hospital Training School for Technicians.

New Jersey

The meetings of the New Jersey Society of Clinical Laboratory Technicians are held on the third Friday of the month. Those held in Newark will be at the North Jersey Academy of Medicine. The officers are as follows: President, Cecil H. Gowen, M.T.; Vice-President, Lallie Lancaster, L.T.; Secretary, Phyllis Stanley, M.T.; Treasurer, Elizabeth Lockwood, L.T.; Executive Committee, Margaret D. Brown, L.T., Pauline Holbert, L.T., Marjorie Edsten, L.T.; Program Chairman, Frieda Ward, L.T.; Membership Chairman, Shiela Stewart, L.T.; Publicity Chairman, Gertrude Jacobus, L.T.

At the October meeting three members reported on the last convention of the A. S. C. L.T., each member presenting the events of one day. There was a discussion of the changes made by the Registry relative to the title of technicians. Paul C. Brown, M.T., gave a resumé of his paper on Phosphatase and Phosphorus as given at the convention.

The November meeting, which was planned by Margaret Kirby, was held in the auditorium of the Jersey City Medical Center, and Dr. St. George gave an interesting discussion on Virus Diseases. Following his paper, there was an inspection tour of the Medical Center.

A paper on Arthritis was presented by Cecil H. Gowen, M.T., at the December meeting which was held at the home of Frank Scalera and the office of Dr. L. W. Brown, after which there was a holiday party.

Ohio

The Ohio Society of Clinical Laboratory Technicians held their second annual State Convention December 4, 1935, at the Onesto Hotel, Canton, Ohio.

The following program was presented: 1:30—Registration. 2:00—Opening Prayer, Rev. J. L. Bardon, St. Peters Church, Canton; Address of Welcome, Dr. F. M. Sayre, Health Commissioner, Canton; Business Meeting and Election of Officers. 3:00—"Demonstration of New Appliances for Kahn Test," Mr. Howard Koch, M.S., Fisher Scientific Co., Pittsburgh, Pa. "The Laboratory as a Teaching Unit," Dr. E. L. Saylor, Pathologist, City Hospital, Akron. Results of the Election. Adjournment. 6:30—Dinner, The Onesto Hotel, Dr. F. C. Potter, of Akron, Toastmaster. 8:00—"Hematology," Dr. Russell Haden of the Cleveland Clinic.

The newly elected officers are: President, Alice Finnin, L.T., Akron; Vice-President, Marjorie Wise, L.T., Canton; Secretary, Martha Andes, L.T., Akron; Treasurer, Ruth Koons, L.T., Barberton; Executive Committee, Sister M. Inez Weaver, L.T., Canton.

Pennsylvania

The Society of Clinical Laboratory Technicians of the Philadelphia District held its second annual dinner at the Rittenhouse Hotel in Philadelphia, Pa., Saturday, October 26, 1935.

Among the speakers for the evening were Dr. Fred Boerner, toastmaster; Dr. Frank Konzelman, Frieda Ward, former president, and Phyllis Stanley, counsellor of the A. S. C. L. T., for the Northeastern States.

Texas

The Texas Society of Clinical Laboratory Technicians held their annual state meeting in Houston, Texas. Headquarters: Rice Hotel.

The meeting was called to order at 9:30 a. m. October 11, 1935, in Parlor C of the Rice Hotel, with the president, H. A. Bardwell of San Antonio, presiding.

Dr. Harry G. Knowles of Houston was unable to be with us for the invocation. Mr. Pierson, representative of Mayor Holcombe of Houston, gave the welcome address. The response by the President, H. A. Bardwell. The minutes of the first annual meeting of the Society in San Antonio, were read. An address on "The History of Registry and Classification of Laboratory Technicians" was given by President H. A. Bardwell. A report was given by Pauline S. Dimmitt, first vice-president, on "The Affiliation of the State Society with the American Society."

The following telegrams were read by the Secretary: (1) Greetings from the Board of Registry of the American Society of Clinical

Pathologists. (2) The Texas Hotel, Fort Worth, Texas, invites the Society to meet in Fort Worth in 1936. (3) Best wishes for a successful meeting from Sister Stella, Fort Worth.

A Round Table Luncheon with discussions of the Technicians' Problems was held in the Banquet Room at the Rice Hotel with Drs. H. A. Braden and H. E. Braun presiding.

Second Session, Parlor C, Rice Hotel, Houston, Texas, 2:30 p. m. The afternoon session was called to order at 2:30, October 11, 1935, with Pauline S. Dimmett presiding and the following program was presented:

"The Erythrocyte Sedimentation Rate: Its Clinical Evaluation," by Dr. J. Walter Torbett, Jr. "Intestinal Parasites," Asa Chandler, M.S., Ph.D. "Problems in Agglutination Tests," Annette Fillius, B.A., L.T. "Interpretations of Urinalysis," Dr. John Alvarez.

Saturday, October 12, 1935—The third session was called to order at 9 a. m. in Parlor C of the Rice Hotel with Edwin Murphy presiding, and the following program was presented:

"Typhus Fever," Dr. S. W. Bohls, Austin, Texas. "Infectious Mono-nucleosis; Hematological and Serological," by Dr. Wm. L. Marr. "Blood Pictures in Leukemias," Y. C. Smith, B.A., Ph.G. "Common Technical Difficulties and Errors in Blood Chemistry," by Dr. M. Bodansky, Galveston, Texas. "The Use of Tribondeau's Stain for Spirocheate Pallida," by Eugene F. Snapp, B.S., L.T.

Discussion of the paper by Dr. Braun.

The fourth session was called to order at 2 p. m. in Parlor C of the Rice Hotel with Mr. Bardwell, President, presiding, and the following program was presented:

"Friedman's Modification of the Ascheim-Zondek Test," by Ellen Wellensick, B.S. "Trichimonas Vaginalis," Dr. Carl Karnaky.

Discussions for the convention city for 1936 followed. Marlin, Texas, was selected.

The President then called for nominations for offices, and the following results were obtained: President, Pauline S. Dimmett of Sherman; President-elect, Ida F. Levinson of Houston; Vice-President, Hazel Bennett of Marlin; Secretary, Anna Lou Smith of Fort Worth; Treasurer, Geo. T. Thomas of Beaumont. Executive Committee: Sister Stella, Fort Worth; C. M. Pitts, Austin; Leonora Plowden, Houston; Annette Fillius, Brownsville; Marion Baker, Wichita Falls; Nelda Childers, Orange.

BOOK REVIEWS

RATS, LICE AND HISTORY. by Hans Zinsser. Little, Brown and Co., Publishers, Boston, 1935. Cloth, pp. 301. Price \$2.75.

It has been said that the only real reason for writing a book is because one wants to, and the fact that the author so obviously enjoys his rôle as the biographer of typhus fever, certainly contributes greatly to the delight of the reader of this book.

The biography is in the best Freudian style but we may forgive its rather broad interpretations because Dr. Zinsser employs only the strictest scientific methods throughout.

All students of epidemiology, bacteriology, and immunology should read it and it should find a place in the libraries of all those interested in the laboratory.

The book can be easily consumed in an evening for it is as good reading as Sherlock Holmes, but Dr. Zinsser remains as accurate as in his sterner and more forbidding "Textbook of Bacteriology."

A TEXTBOOK OF BIOCHEMISTRY, edited by Benjamin Harrow, Ph.D., Associate Professor of Chemistry, the City College, College of the City of New York and Carl P. Sherwin, M.D., Sc.D., Dr.P.H., LL.D., member of the Staff of St. Vincent's Hospital and French Hospital, New York City. W. B. Saunders, Publishers, 1935. Cloth, 797 pages, Price \$6.00.

This book, just released from the press, will fill a place between Hawk and Bergheim and Howell on the laboratory book shelf. This place has been vacant for a long time.

It is a compendium of monographs by thirty eminent authorities from both sides of the Atlantic. Only five of the contributors are doctors of medicine. For this reason the interpretation and practical applications of biochemical procedures receive scant attention. Also for this reason, the approach to these problems is from the "pure science" angle, and the text is much better chemistry than that contained in the usual applied biochemistry or physiology.

The book would serve as a very modern physiology. It is introduced by a chapter on "The Cell" by Dr. Robert Chambers. Then comes the chemistry of carbohydrates, protein and fats. Next nutri-

tion and enzymes are dealt with. Following this come the chapters on the metabolism of certain classes of substances in the human body, preceded by discussions on oxidation and reduction processes and respiratory metabolism. Finally the chemistry of certain of the tissues is discussed. Numerous chapters on other subjects are interposed. The chapters on the biochemistry of bacteria, yeast and moulds, on animal pigments and on hormones will prove very interesting to any worker in a general laboratory.

The chapter on Immunochemistry is particularly apt. Many of us have never had the faintest inkling of what immunity was all about since they cut Erlich's old diagram out of the bacteriology. The newer concepts of "antigen", "antibody" and precipitation and agglutination reactions are explained so well that you think you've had a course in "How to be an Immunologist in Ten Easy Lessons." Dr. Rowntree's contribution on the Function of Water in the Organism, Dr. Bloor's treatise on Lipoid Metabolism and the discussion of the coagulation of the blood are particularly satisfactory.

Notwithstanding the vast amount of material contained in the book and the multiplicity of the authorship, the text is neither exhaustive nor exhausting. Ample bibliographies are supplied by each author. In spite of the individual method of each author in presenting his material, the style is excellent throughout. The chapters have been well edited and arranged and the sequence is disturbed as little as if the book had been written by the same person throughout.

BOOKS RECEIVED

PRINCIPLES OF BACTERIOLOGY, by Arthur E. Eisenberg, A.B., M.D., Director of Laboratories, Sydenham Hospital, New York, Member of the Pathological Society of New York, and Mabel F. Huntly, R.N., M.A., Director of Nursing, Wesson Memorial Hospital, Springfield Mass. Sixth Edition, 1935. C. V. Mosby Co., St. Louis, publishers. Pp. 378, Cloth. Price \$2.75.

PRACTICAL HEMATOLOGICAL DIAGNOSIS, by O. H. Pepper, M.D., Professor of Clinical Medicine, University of Pennsylvania, Assistant Chief of the Medical Clinic, Hospital of the University, and David L. Farley, M.D., Physician to the Pennsylvania Hospital, Philadelphia, and the Cooper Hospital, Camden, N. J., Associate in Medicine at the University of Pennsylvania. W. B. Saunders, Philadelphia, publishers, 1934. Cloth, pp. 562. Price \$6.00.

A TEXTBOOK OF BACTERIOLOGY, by Thurman B. Rice, A.M., M.D., Professor of Bacteriology and Public Health at the Indiana School of Medicine. W. B. Saunders, Philadelphia, publishers, 1935. Pp. 551, Cloth. Price \$5.00.

A TEXTBOOK OF LABORATORY DIAGNOSIS with Clinical Applications for Practitioners and Students of Medicine. By Edwin E. Osgood, M.A., M.D., Assistant Professor of Medicine and Biochemistry, University of Oregon, School of Medicine, Portland, Oregon. Second Edition, Revised, 1935. P. Blakiston's Sons & Co., Philadelphia, publishers. Pp. 585, Washable Cloth. Price \$6.00.

ABSTRACTS


A RAPID METHOD FOR THE IDENTIFICATION OF DIPHThERIA BACILLI: Brahdy, Lenarsky, Smith and Gaffney, J. A. M. A., Vol. 104:21, May 25, 1935.

Although written for the busy clinician this article should be of even greater value to the technician. The authors use sterile cotton swabs impregnated with undiluted, unheated horse serum to which no preservative has been added. The swabs are squeezed against the sides of the tube to remove excess serum, lightly heated over a flame to obtain surface coagulation and then used to take nose and throat cultures in the usual manner. Instead of implanting on a culture medium the swabs are put in dry sterile tubes, placed in an incubator and examined at the end of two and four hours. They found the results to be much quicker, more often positive, and more uniform results in the morphological identification of the diphtheria bacilli. Exact figures are given in the tables.

THE STABILITY OF SUGAR IN THE CEREBROSPINAL FLUID: Purcell G. Schule, M.D., Jour. Lab. & Clin. Med., 20:7, 1935.

This report will satisfy some of us who are called upon to perform spinal fluid sugars a day or more after the specimen has been collected. Dr. Schule has proved that the quantity of sugar in cerebrospinal fluid kept under sterile conditions at 10° C. remains unchanged for a period of at least 21 days.

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THE VALUE OF THE TAKATA AND ARA REACTION AS A DIAGNOSTIC AND PROGNOSTIC AID IN CIRRHOSIS OF THE LIVER: Alex B. Ragins, M.D., Jour. Lab. & Clin. Med., 20:9, 1935.

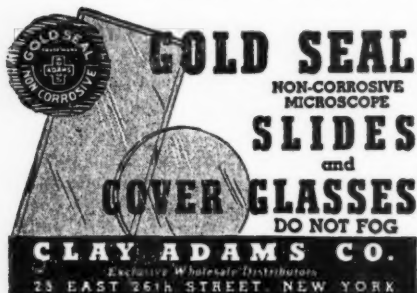
The author presents the method of performing the Takata and Ara Reaction (T. A. R.) and explains the interpretation of the results: Summary:

1. Two hundred and seventy-six cases with the T. A. R. have been studied.
2. That this is of value in cases of decompensated liver conditions due to cirrhosis is without doubt. For latent liver damage the test is not entirely reliable.
3. The T. A. R. is positive in 98% of the cases of cirrhosis of the liver.
4. The simplicity with which the test is carried out warrants its use in hospital and office practice as both a diagnostic and prognostic aid in cases of cirrhosis of the liver.
5. Liver impairment occurs in hyperthyroidism, as shown by the T. A. R.

THE EVALUATION OF SERODIAGNOSTIC TESTS FOR SYPHILIS IN THE UNITED STATES: Report of Results: Cummings, et al, J. A. M. A., Special Article, Vol. 104: 23, June 8, 1935.

The authors do not comment on the relative value of the various tests studied but simply present their figures and tables, leaving the reader to draw his own conclusions. The reports cannot be further condensed so must be studied in full. They should be of special interest to technicians performing serologic tests of any kind for syphilis. For further information the reader is invited to write to Surgeon General Cummings, U. S. Public Health Service, Washington, D. C.

The editorial comment on the above report given in the same issue of the Journal, page 2099, should be read in conjunction with



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Bizzozero discovered the blood platelets in 1882.

Walther Flemming investigated cell division in 1882.

the survey. One point of interest, for instance, is the recommendation that reports should be simply "positive," "doubtful," or "negative," also that at least on blood specimens the flocculation tests rate higher in the exclusion of syphilis and rate as well if not better in the diagnosis of syphilis than the more complex complement fixation test, but the warning against a positive report with only one test if the highly sensitive flocculation tests are used.

BLOOD GLUCOSE CLEARANCE: Studies in Normal and Diabetic Persons: Richard M. McKean, M.D., et al., Amer. Jour. Med. Sc., 189:5, 1935.

A new method of performing the glucose tolerance test. A vein is entered, a fasting blood specimen taken and glucose to the amount of 0.2 gm. per kg. body weight is injected in exactly 1½ minutes. Within the next three minutes a vein in the opposite arm is entered and specimens of blood are collected exactly 3, 4, 5, 10, and 15 minutes after the end of the glucose injection. The needle is not removed for the several specimens as a tube with a two-way stop-cock is attached to the intravenous needle.

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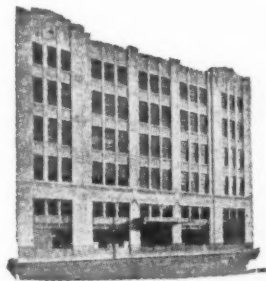
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